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Neural Adaptations in the Visual Pathway of Certain Heliconiine Butterflies, and Related Forms, to Variations in Wing Coloration[1]

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(Figures 1-8)

INTRODUCTION

THE EXPERIMENTS which form the basis of this paper were stimulated by the well-known responsiveness of butterflies to visual stimuli of various colors. Behavioral observations of this phenomenon include those of Eltringham (1933), Ilse (1937). Magnus (1956), and most particularly those of Crane (1955, 1957).

Crane's demonstration of the responsiveness of *Heliconius erato* to orange-red stimuli in feeding and courtship behavior prompted a series of experiments designed to determine the physiological basis of this special sensitivity to long wavelengths. These experiments (Swihart, 1963, 1964, 1965) have demonstrated that: (1) the visual system of *H. erato* includes at least two types of receptors, one maximally sensitive to blue-green and the other peaking in the red (ca. 620 mμ); (2) the receptors interact with each other producing an electroretinogram (ERG) waveform with a distinct color component; (3) specific neural pathways are associated with these receptors. Recordings from the internal chiasma, medulla interna, and the vicinity of the optic nerve demonstrate a spectral sensitivity which indicates a particularly close association between the red receptors and the main pathway of information to the brain.

The behavioral sensitivity of this species seems, therefore, to be related not to any special modification of the receptors, but rather to the development of pathways which "selected" the output from those receptors which transduced information with special biological significance.

The basic hypothesis emanating from these observations was that there was a selective advantage in developing neural mechanisms which demonstrate disproportionate sensitivity to the basic wing coloration, presumably because of the role played by such colors in releasing courtship behavior. This hypothesis invited testing by

conducting experiments on forms other than *erato,* which possessed different wing coloration, to determine how general this mechanism might be, and the degree of adaptive flexibility afforded by such a system. This paper reports the preliminary results of such a comparative study.

Methods and Materials

Standard electrophysiological techniques were employed. Intact organisms were rigidly mounted with plasticene as previously described (Swihart, 1963, 1964). Evoked potentials were recorded from the optic lobe with a semi-micro (ca. 500 kohm), 3M KCl filled, glass electrode. On the basis of previous experiments (Swihart, 1965), a technique was developed for placing the electrode almost directly into the medulla interna, via a minute hole in the posterior (occiput) region of the head. Usually only very small additional movements of the electrode were required to obtain the long-latency, negative polarity, response characteristic of the medulla interna (see fig. 15, Swihart, 1965).

In order to ascertain that the preparation was continuing to yield "normal" responses to photostimulation, ERGs were recorded simultaneously with a sub-corneal steel electrode. Experiments were terminated if there was any change in the ERG waveform.

Potentials were amplified with Grass P6 D.C. amplifiers, displayed on a Tektronix four beam 564 oscilloscope, and photographed with a Grass C-4 camera for subsequent measurement.

Photostimulation was accomplished with a laboratory constructed stimulator which, automatically, sequentially introduced a series of 15 narrow-band interference filters into an optical system, and provided a 100 msec. stimulus at each wavelength, at a preset and constant interval, usually about one minute. The stimulus duration was chosen as the shortest which would ensure production of all the components of the ERG (Swihart, 1964).

Preparations were aligned so that the stimulating beam, focused by a microscope objective, illuminated nearly an entire eye. The beam axis was perpendicular to the center of the cornea. Histological studies which have been conducted on a number of the forms selected for this study *(Morpho, Agraulis, H. sarae,* and *H. erato),* and physiological experiments involving the stimulation of small portions of the eye (of *H. erato),* have not demonstrated any anatomical or physiological differentiation between various regions of the eye.

Peak transmission points of the interference filters were fairly uniformly spaced throughout the visible spectrum from 404 mμ to 709 mμ.

Through the use of a specially-ground, color-compensating filter, and sandwiched gelatin filters, the stimulus energy at each wavelength was held constant ($\pm$ 20%) as determined with an Eppley thermopile.

Equipment was not available for making small, calibrated adjustments in stimulus intensity, hence "spectral sensitivity" (threshold energy) curves were not attempted, rather the electrical magnitude of the response to a standard stimulus of about 5×10^3 microwatts/cm^2 was determined.

Spectral efficiency curves were constructed by running through the series of 15 filters three times for each preparation. The magnitude of the response to a particular stimulus was evaluated as a percentage of the largest response to any filter in that particular series. The percentage responses to the three successive series were then averaged to produce the curve for a single preparation. The technique of recording the amplitude of the response, seemed justified since the magnitude of the medulla interna response demonstrates a nearly linear relationship to the log of the intensity of stimulation, within the range of intensities studied. In this range, a tenfold increase in white light intensity, produces an increase in response magnitude of about 30%.

Experimental material was normally captured in the wild and maintained in large outdoor insectaries. This technique permitted the selection and testing of only healthy animals.

Wing spectral reflectance characteristics were measured with a Bausch & Lomb "Spectronic 20" spectrophotometer with reflectance attachment. This equipment measures the reflectance of an area approximately 2 mm x 8 mm. Only specimens which appeared to be newly emerged were used for such determinations.

It will doubtlessly be noted that the number of specimens of each species investigated is frequently very small. It is hoped that this will be understood as related to certain problems which were encountered, including: (1) the physical difficulty of capturing healthy specimens of certain elusive and rare species, notably *Philaethria* and *Morpho;* (2) the extremely delicate nature of these organisms which results in their entering a state of "shock" if handled roughly. This condition is evidenced by abnormal behavior, including strong positive phototaxis, and highly aberrant evoked potentials, usually accompanied by very large (ca. 5 mV) low-frequency spontaneous discharges; (3) the necessity of limiting the observations to only those individuals which produced strong "day" type electroretinograms (Swihart, 1963); (4) the problems in placing the micro-electrode into the

intact organism in such a manner that it reaches the very limited region near the medulla interna, which yields the long-latency negative potentials, without inducing operative trauma, and its resultant massive spontaneous activity.

Six species of butterflies were selected for this study. All belong to the family Nymphalidae. Four are members of the subfamily Heliconiinae, while the Morphinae is represented by one form *(Morpho)*, and the Nymphalinae by *Victorina*.

Studies were begun on *Heliconius sarae* because of its fairly close relationship with the subject of previous experiments, *H. erato*. In Trinidad, *erato* is a small black butterfly (2½″ wingspread) with bright red spots on the forewings. *H. sarae* is, in many respects, a very similar butterfly, with the most obvious difference being the substitution of a pair of bright yellow bands on each forewing. In addition there is a moderately strong blue irridescence on the hind wings.

Heliconius ricini was chosen because it offered an "intermediate" color pattern to *sarae* and *erato*. In *ricini,* the black "base" color of the wings is broken by forewing yellow bands (as in *sarae*), but a large red hindwing spot is also present. *H. sarae* and *ricini* are the most closely related of the forms tested. Both belong to the same species-group as discussed by Emsley (1965).

The third form chosen, *Agraulis vanillae,* was selected because its color pattern is similar to that of a number of other primitive Heliconiinae. That is to say, the wings are predominantly bright orange in color.

Even a cursory glance at the variety of wing color patterns characteristic of the Heliconiinae demonstrates a single basic plan. This is a long-wavelength color with a contrasting dark brown or black. It can be seen that the aforementioned forms are representative of this basic pattern. However, for purposes of investigating the physiological adaptations of the visual pathway, it seemed desirable to investigate forms with an unusual wing coloration. Initially, it was hoped that studies could be made on the rare, predominantly green heliconiine, *Philaethria dido;* however, only a single healthy specimen of this species was obtained, and observations were extended to the very similarly marked nymphalid. *Victorina steneles.*

Finally, to further extend the variety of wing colors, observations were made on the blue-winged *Morpho peleides.*

The results of the observations on each species will be considered separately.

RESULTS

Heliconius sarae thamar Hubner

A total of eight individuals that were tested yielded acceptable responses, i.e., long-latency negative potentials. A ninth individual producing such responses became erratic before the experiment was completed; however, its performance in the early stages of the experiment gave a clear indication of its sensitivity.

It was found that the spectral efficiency curves produced by these individuals were far from being identical to each other. Examination of the curves revealed that they could be separated into two distinct types, with each category being fairly homogeneous. Figures 1 and 2 show these two types of curves. Four individuals demonstrated a sensitivity peak in the orange-red portion of the spectrum (ca. 620 mμ); the remainder were maximally sensitive to green.

In spite of the small sample size, the differences between these two curves is so great that there is no statistical basis for doubting that they represent two distinct populations. Thus, for example, one obtains by comparing the percentage responses at a single wavelength (523 mμ), at value of 4.78, with a n = 7[*]. The probability that the responses represent different populations is thus greater than 0.995. If this analysis is carried to additional points on the spectra, no reasonable doubt can remain.

The red-sensitive curve peaks at 620 mμ, and in this respect is very much like the responses recorded from *erato*. However, there is a difference between the two forms in their sensitivity to shorter wavelengths. Thus, the response of *sarae* to stimulation with short wavelengths is, on the average, considerably greater than *erato* (Fig. 2).

Figure 3 shows the spectral reflectance curves of the *erato* red pigment, and the *sarae* yellow. It can be seen that the yellow color could actually be described as "blue negative," since it actually reflects about the same amount of red as does the *erato* pigment. If one, therefore, considers the difference between these colors in terms of a greater reflectance of mid and shorter wavelengths, there is a parallel in the differences between the reflectance and spectral efficiency curves in the two forms.

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[*] where

$$n = \frac{\left(\dfrac{s_1^2}{N_1} + \dfrac{s_2^2}{N_2}\right)^2}{\left(\dfrac{s_1^2}{N_1}\right)^2 \dfrac{1}{N_1 + 1} + \left(\dfrac{s_2^2}{N_2}\right)^2 \dfrac{1}{N_2 + 1}} - 2$$

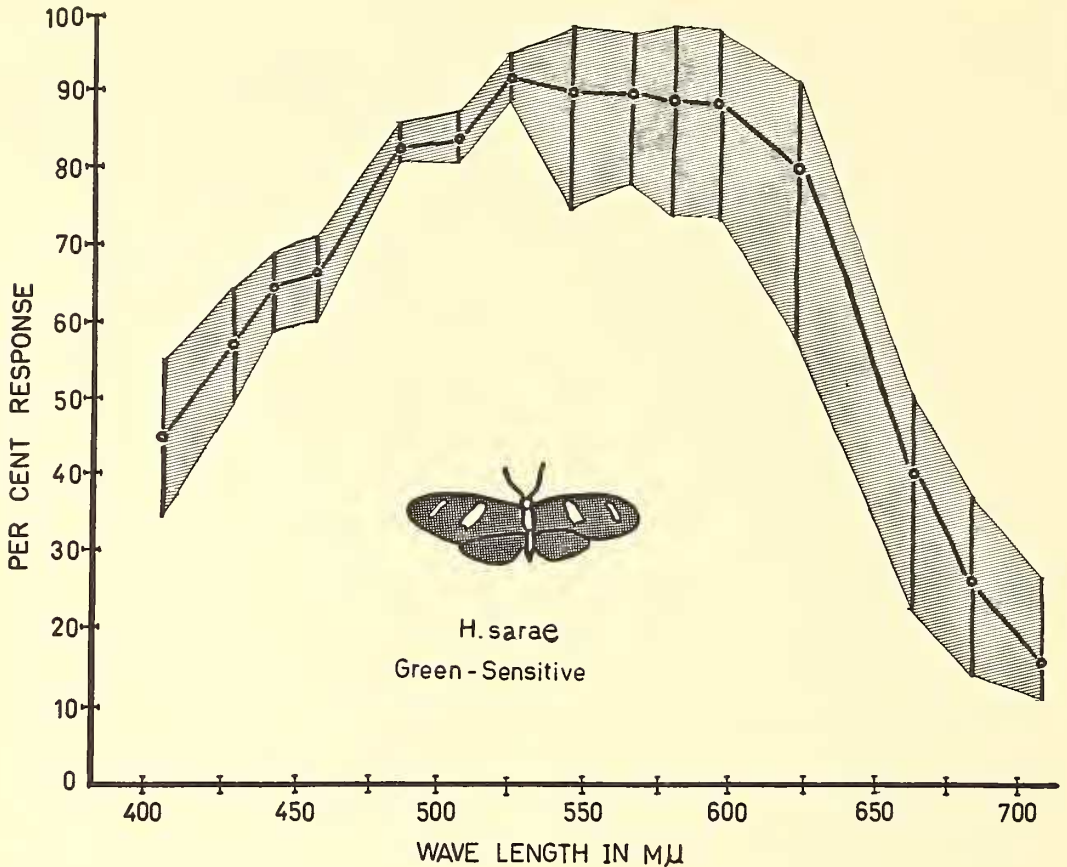which is an approximation of a longer formula given by Welch (1947).

FIGURE 1.

One of the two types of spectral efficiency curves found to be characteristic of *Heliconius sarae*. Measurements were based upon the magnitude of long-latency, summated potentials of negative polarity in the vicinity of the medulla interna. See text for details of technique. Vertical bars indicate the limits of observed variability between individuals, i.e., the maximum and minimum responses to any given wavelength. Illustration is a semi-diagramatic representation of the dorsal surface; unshaded portions correspond to the areas of yellow pigmentation. Shaded area represents the distribution of near black wing pigmentation. Unless otherwise noted, all subsequent diagrams are to the same scale.

The other type of spectral efficiency curve was recorded with the same frequency as the red-sensitive one (four of each, with the previously mentioned ninth being red sensitive). It would appear that the sensitivity of these organisms was largely determined by the green-sensitive receptor system with a slight and variable skew produced by the presence of the red system.

At this time it is impossible to identify the factors which contribute to the differences between these two types of individuals. All the organisms were tested at approximately the same time of day (late morning), and demonstrated similar "day" type electroretinograms. Three males and one female yielded the red-sensitive curve, while three females and one

male produced the green curve. The age of the individuals was not known since they were all wild caught specimens.

It should be remembered, however, that behavioral observations on butterflies have demonstrated rather dramatic shifts in the colors to which they will respond, depending upon their physiological state. Thus, Ilse (1937) showed that while *Pieris brassicae* selected red, yellow or blue colors in its feeding behavior, it became specially responsive to the color green when involved in egg-laying behavior. It may well be that such shifts in spectral sensitivity represent a measurable index of the rather abstract concept of "physiological state."

Heliconius ricini (L.)

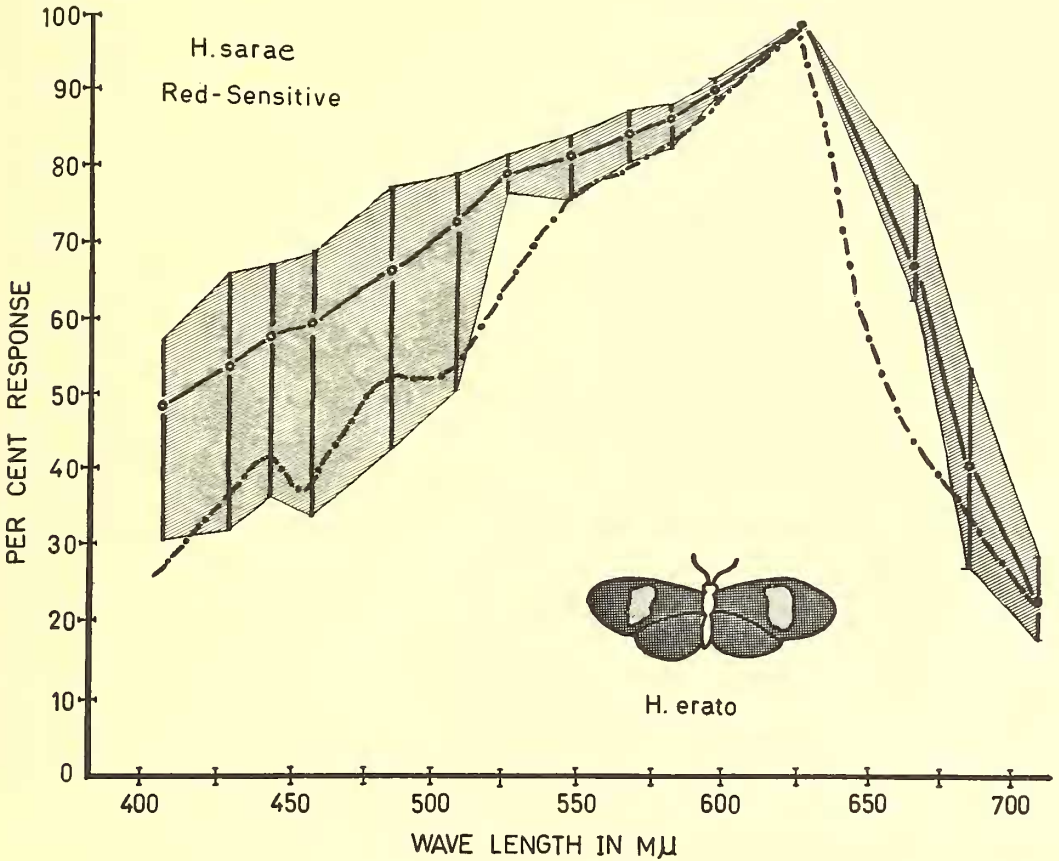A total of eight specimens of this species pro-

FIGURE 2.

Alternate type of spectral efficiency curve that may be recorded from *Heliconius sarae* which demonstrates far greater sensitivity to long wavelengths than does the curve in Figure 1. Also included in the figure, for purposes of comparison, is the comparable average spectral efficiency curve recorded from four individuals of the species *Heliconius erato* (dot-dash-line). Also figured is the distribution of red pigmention in the forewings of *H. erato*.

duced acceptable responses. In spite of the fact that the wing coloration of this form can be considered to be intermediate between *sarae* and *erato* (i.e., has both red and yellow markings), the responses were entirely similar to *sarae*. Thus three of the individuals yielded spectra peaking in the red (Fig. 4) (620 mμ), with no significant differences from the *sarae* red-sensitive curve. One of the specimens tested was most interesting in that the nature of its response changed during the course of the experiments. The responses to the initial filter series indicated a maximum sensitivity to red. A gradual change took place, without any apparent change in the waveform of the response, so that by the time the organism was stimulated by a fourth series of filters, the response was plainly maximally sensitive to green light.

The remainder of the organisms tested produced the non-specific green-sensitive curve.

Agraulis vanillae vanillae (L.)

Five specimens of this primarily orange-colored butterfly produced acceptable responses. The averaged responses of these individuals produced a curve which is distinctively different from any which had been previously recorded (Fig. 5). In many respects, this spectrum can be considered to be entirely intermediate between the two *sarae* spectra. Thus, *Agraulis* demonstrates a curve peaking in the orange, less sensitivity to short wavelengths than the *sarae* green curve, and less sensitivity to long wavelengths than the *sarae* red curve. These differences are so great that there is little or no overlap of even those responses demonstrating the
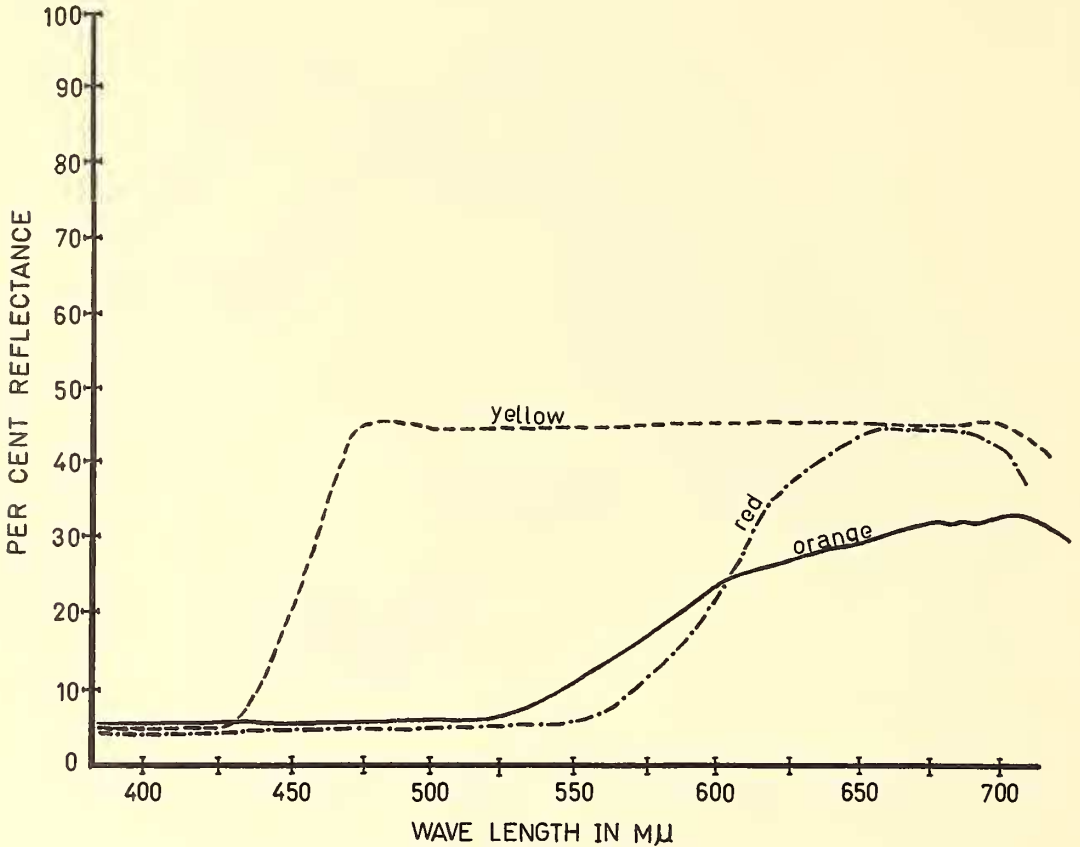
FIGURE 3.

Spectral reflectance characteristics of various heliconiine wing pigments as measured against a magnesium carbonate standard. Intact wings were mounted on a nonreflective backing before being inserted into the spectrophotometer. No differences in reflectance exist between similar appearing areas in the various species, e.g., *erato* and *ricini* red are identical.

greatest variance from the mean in most of the aforementioned portions of the spectrum.

It will be noted that, like *erato,* only a single type of response was recorded from this species.

Philaethria dido dido Clerck and *Victorina steneles* (L.)

It was hoped that observations could be made on the green-winged heliconiine *Philaethria* as it would provide a form with a markedly different wing coloration. Unfortunately, not a single wild specimen could be obtained. Several eggs were located, however, and a single healthy specimen was raised in the laboratory. Testing of this individual did indeed produce responses showing a distinctive peak in the green. Regrettably, no additional specimens were available to confirm these observations. Because of the shortage of *Philaethria,* it was decided to make observations on the remarkably similar nympha-

lid *Victorina.* These forms are so similar that they can be easily confused. The resemblance is much more profound than mere superficial appearance. Both forms have developed their green coloration by a most interesting technique. In the green areas of the wings, the scales are either much reduced, or missing, and the pigmentation is within the wing membranes. The coloration itself appears to be due to the presence of two pigments. One of these absorbs in the blue (i.e., appears yellow) and can be extracted with ether, leaving an insoluble blue compound behind (Fig. 6). It seems likely that this unusual wing coloration is due to haemolymph pigments (Hackman, 1952) since *Victorina*'s blood and eggs are both bright grassgreen.

Four specimens of *Victorina* were tested and found to resemble *Philaethria* in visual mechanisms as well as in pigmentation. Figure 7 shows
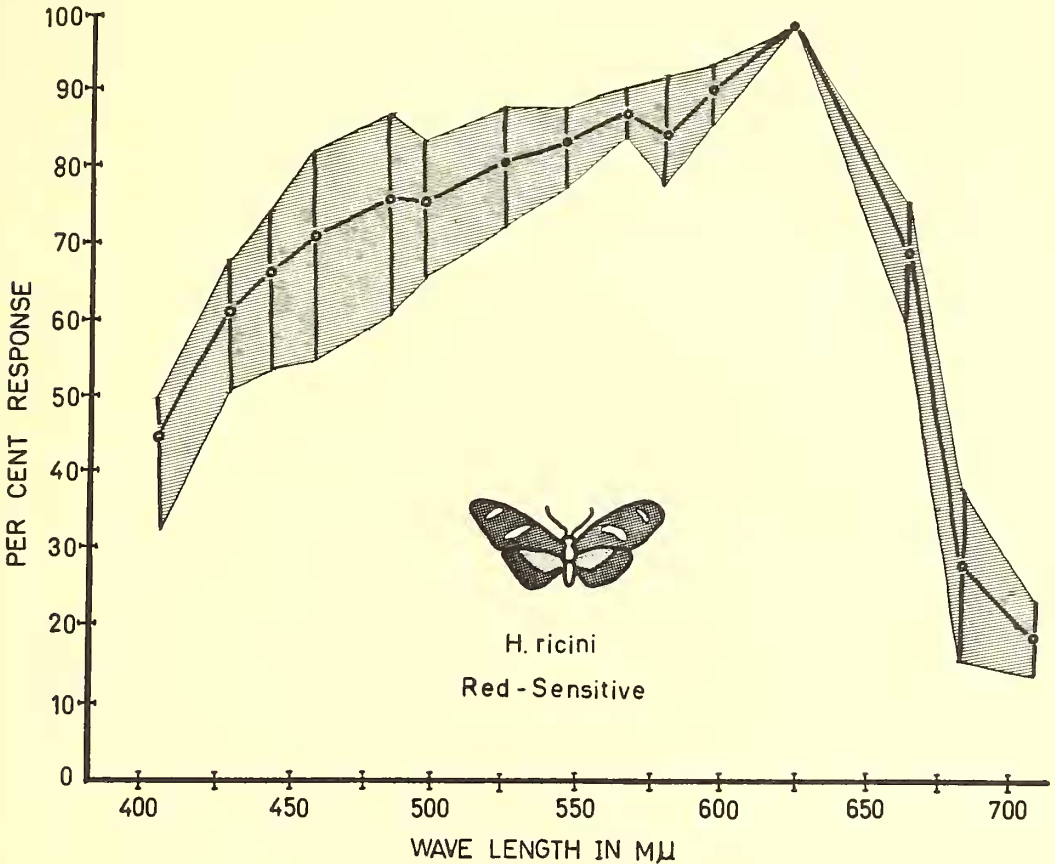
FIGURE 4.

One of the two spectral efficiency curves characteristic of *Heliconius ricini*. Note the great similarity to Figure 2. The other type of curve is similar to the *sarae* "green-sensitive" curve illustrated in Figure 1. Unshaded areas of illustration correspond to the distribution of the yellow pigment, while the lightly shaded area represents the hind-wing red spots.

the luminosity curve produced by these individuals which clearly peaks at about 565 mμ. There can be no doubt that this curve is distinctly different from the green-sensitive curve of *sarae*. At virtually every wavelength longer than the 565 mμ maximum, the *Victorina* responses are so greatly reduced that there is very little, if any, overlap with even those *sarae* showing the greatest deviation from the mean.

The curve recorded from the single *Philaethria* was entirely similar to that produced by *Victorina,* and fell well within the limits of *Victorina* variability.

Morpho peleides insularis Fruhstorfer

It was considered advisable to make tests on a form with a primarily blue wing coloration. Since there is no such heliconiine in Trinidad, it was decided to utilize the large blue-violet morphine *Morpho peleides*. This butterfly is well known to show a special sensitivity to the color blue. For many years, professional collectors have used this fact to assist in the capture of this elusive species, with its highly prized iridescent physical coloration.

Two specimens of this species were fully tested for spectral efficiency. The electrical responses produced by these organisms were found to be so different from the responses of the heliconiine that direct comparison is difficult. The electroretinograms show none of the complexities, described in detail for *erato* (Swihart, 1964). The waveform consists of a B wave, followed by a uniform, sustained negativity during illumination. All the ERG components usually associated with the red-sensitive receptor, such as the "dip" following the B wave, and "off" effect, are totally absent.
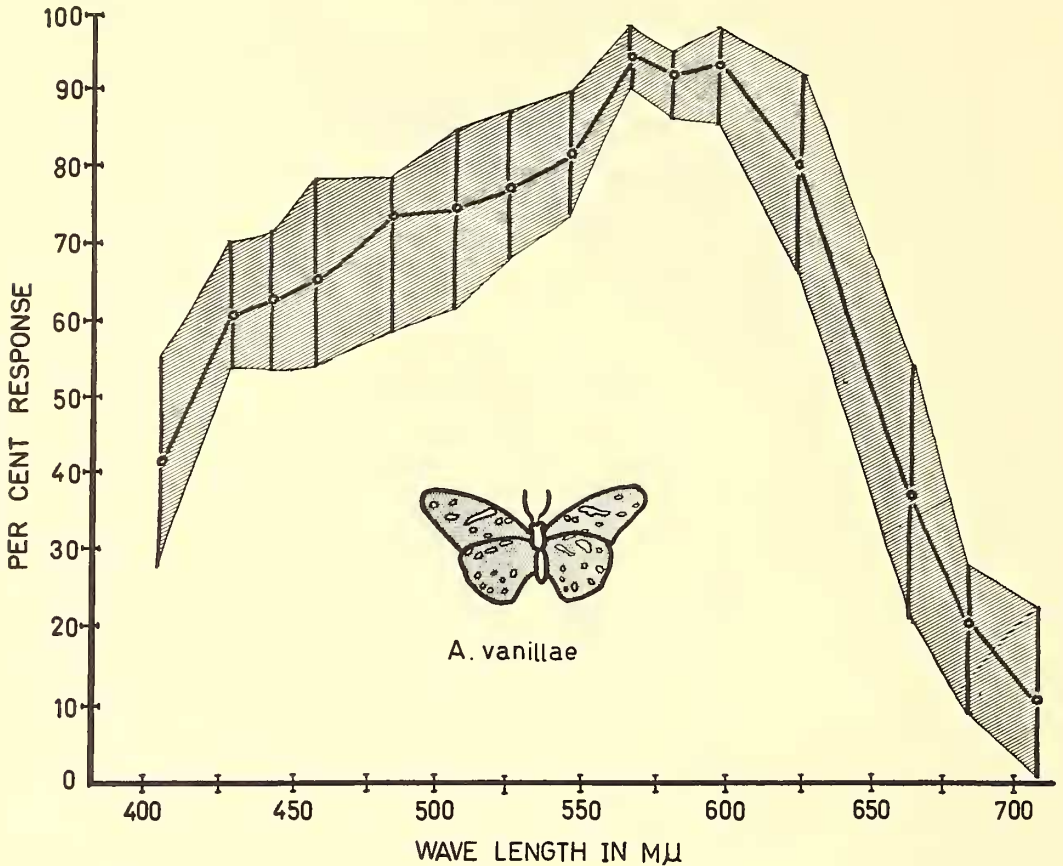
FIGURE 5.

Single type of spectral efficiency curve recorded from five specimens of *Agraulis vanillae*. This species is a nearly uniform orange color (light shading), with numerous small black (unshaded) spots on the wings.

These differences in the ERG waveform are reflected in differences in evoked potentials, as recorded with micro-electrodes. Unlike the heliconiine, it was found that the evoked potentials were generally directly proportional in magnitude to the size of the ERG. The spectral efficiency of these effects, as measured by the ERG B wave magnitude, is indicated by Figure 8. The skew of this curve towards the blue, with its peak at about 485 mμ is both obvious and strikingly different from the curves produced by the heliconiine.

Two additional specimens of this species were tested in the course of work preliminary to the experiments which form the basis of this report. Full spectral efficiency curves, as determined by the standard technique, were not calculated for these individuals. They did, however, appear to demonstrate the same ERG waveform and great sensitivity to short wavelengths.

DISCUSSION

It is unfortunately true that the fundamental mechanisms of color vision remain largely unexplained. This lack of knowledge is particularly apparent in the case of invertebrates, where even the method of coding color information has not been identified as has been done for certain vertebrates (e.g., Wagner *et al.,* 1960; Muntz, 1962).

Recent microelectrode studies (e.g., Horridge *et al.,* 1965) have revealed a vast complexity in discharge patterns in the insect visual pathway. There is, however, some question as to the adequacy of such single fiber techniques to record from those fibers most intimately involved in the highest forms of behavior. There can be no doubt that these techniques tend to select large-diameter fibers. Such neurons have been repeatedly demonstrated to be associated with flight or escape reactions, and hence can hardly be
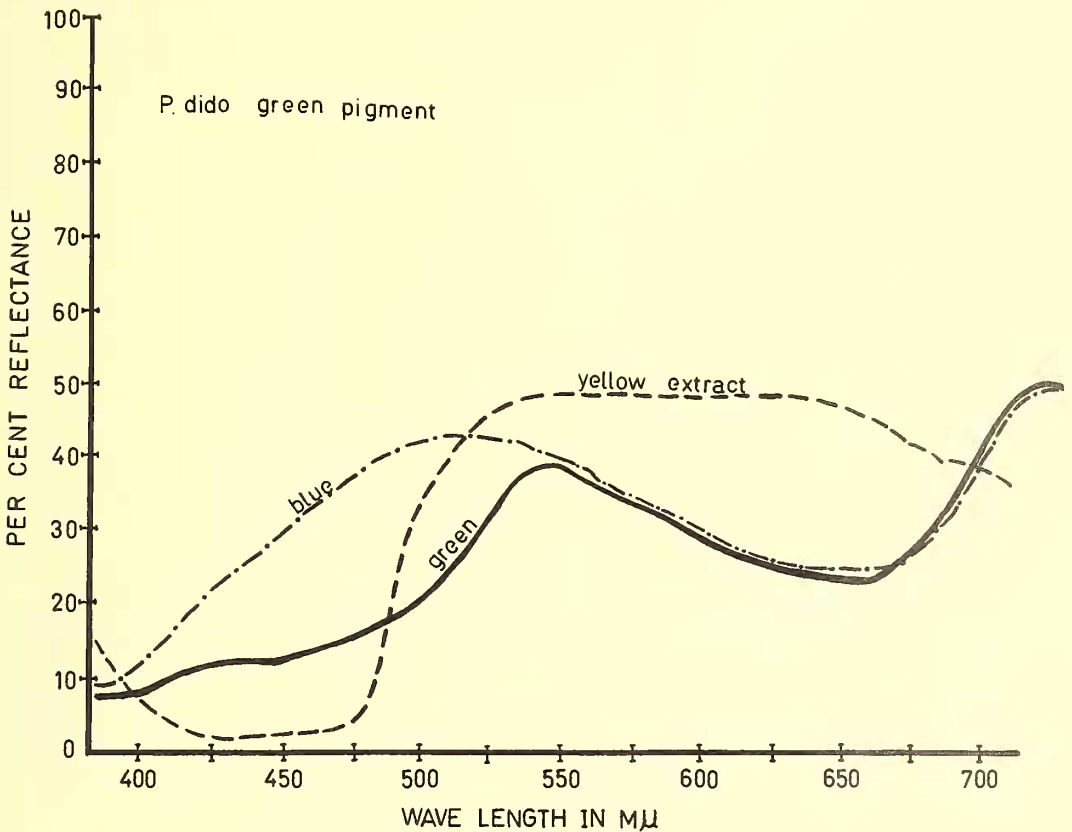
FIGURE 6.

Reflectance characteristics of the green wing areas of *Philaethria dido* and *Victorina steneles*. Since the scales are much reduced, or absent in these regions, they are quite translucent and reflect poorly. Because of this fact, the wing reflectance was measured with a white backing. This technique increased the height of the curve without altering its shape. Solid line illustrates the characteristics of the natural wing coloration. Dot-dashed line indicates the reflectance of the wings after being extracted with ether. Dashed line indicates the transmittance of the yellow-colored extract. The height of this curve was adjusted by plotting $\frac{\% \text{ transmittance.}}{2}$

considered as representative. For these reasons semi-microelectrodes have been employed in the current study, as it seems not unlikely that a "summated" response reflects the nature of the nervous activity with somewhat less bias.

In this connection, it should be remembered that it was demonstrated (Swihart, 1965) that there are fibers with discharge patterns related to the magnitude of such summated potentials. Thus, the curves presented in this report reflect the discharge frequency (and hence the total number of spikes per stimulus of standard duration) of at least some of the neurons in the visual pathway.

Recently, Goldsmith (1965) has given evidence that spectral sensitivity curves derived

from summated (ERG) potentials must be carefully interpreted if screening pigments are present. Such problems are not encountered in the genus *Heliconius,* where the only pigmentation appears to be a nearly black substance, localized in granules within the iris pigment cells.

In the case of *Agraulis* and *Victorina* there is a light orangish pigmentation within the corneal cuticular layer. This pigmentation does not appear to penetrate to the deeper layers of the eye, and hence probably could not produce the effects described for *Musca.* However, the role of such accessory pigments deserves further investigation.

A careful analysis of the techniques employed in these experiments may suggest that they lack
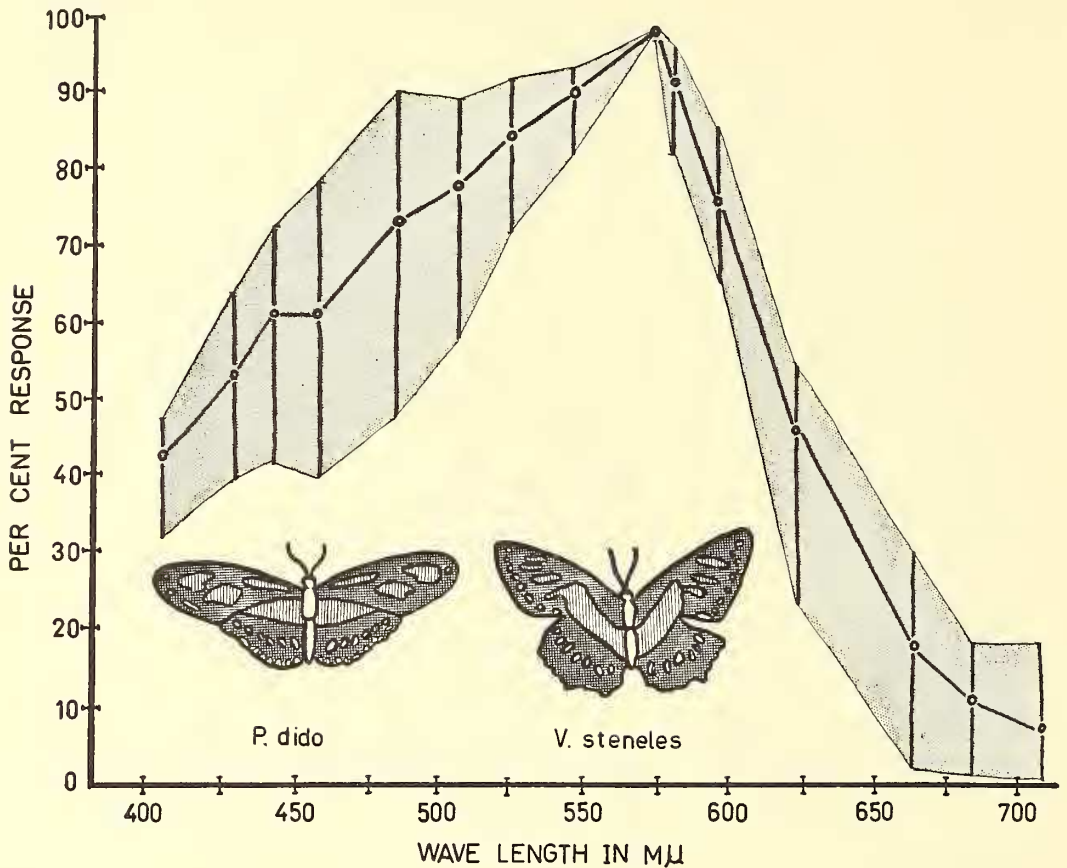
FIGURE 7.

Spectral efficiency curve recorded from four specimens of *Victorina steneles*. The single specimen of *Philaethria dido* demonstrated a similar sensitivity. Lightly shaded areas of illustration represent the distribution of the green coloration, as opposed to the dark brown-blackish pigmentation.

some of the controls commonly employed in the investigation of primary visual events. It must be remembered that this is primarily a comparative study and that the techniques employed on one form were identical to those used on the others. Thus, while it may not be possible to interpret the results as representative of photopigment absorption spectra, they are indicative of real differences which exist between closely related forms.

It is reasonable to enquire as to the origin of these differences.

Analysis of the magnitude of the electroretinograms, in a method analagous to that described in this paper, produces spectral efficiency curves with no significant differences between the various Heliconiinae. In every case the curves resembled that described for *H. erato* (Swihart, 1963). This fact strongly suggests that the varia-

tions between forms cannot be related to differences in the nature of the photopigments.

Alternatively one might suggest that the variations in sensitivity are due to differences in the relative numbers of several different types of receptors, (e.g., *Calliphora;* Autrum & Burkhardt, 1961). However, an explanation based upon such a rigid mechanism seems inconsistent with the type of variability observed in *sarae* and *ricini.*

For such reasons, it seems most reasonable to interpret the observed variations in the summated responses to various colors, as a neural phenomenon.

Turning to a consideration of the spectral efficiency curves themselves, we find a most interesting series in the responses of *Agraulis, sarae* and *erato.* In considering these forms, it is worth noting that casual behavioral observations
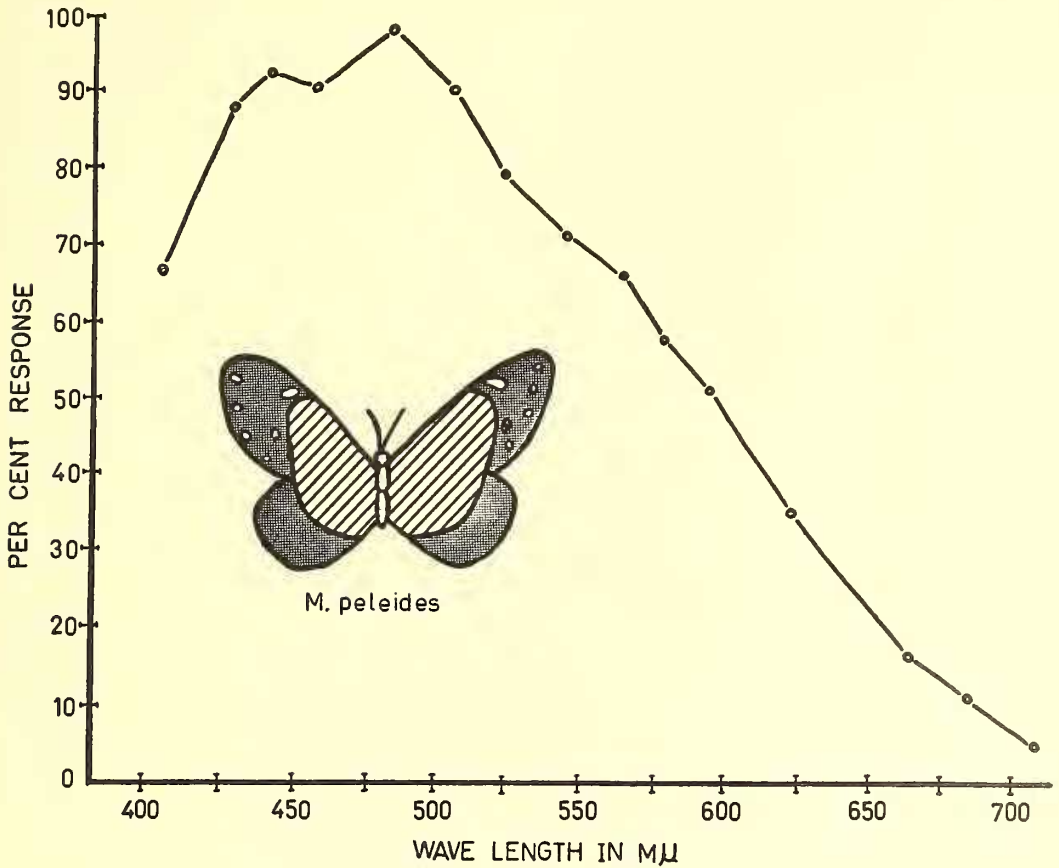
FIGURE 8.
Average spectral efficiency curve derived from two specimens of *Morpho peleides,* as determined by the ERG B-wave magnitude. Very little variability between the individuals was observed. Illustration is at reduced scale. Central (hatched) areas of wings is iridescent blue, with contrasting brown-black margins.

by the author have indicated that consistently stronger behavioral responses to specific colors are produced by the species with the red and orange markings. In the case of *sarae,* attempts to demonstrate a behavioral sensitivity to yellow or red have, as of this time, produced no positive responses in courtship (Crane, pers. com.). This condition would seem to have a physiological correlate in the existence of the two types of spectral efficiency curves recorded from this form, as opposed to the uniformity which characterizes the two other species.

It would appear that considerable significance can be attached to the variability between individuals producing each of the two different *sarae* curves. Each of the curves demonstrates a highly asymmetric distribution of the degree of variability, which would hardly be expected on the basis of a "normal" distribution of variance. The most likely interpretation of these

curves would seem to be that the green-sensitive curve reflects the activity of a visual system responding primarily to the sensitivity of a blue-green sensitive receptor, with a small and variable contribution by a red-sensitive system. On the basis of such an interpretation, the short wavelength portion of this curve (400-525 mμ) would probably reflect a portion of the inherent sensitivity of the receptor system. Conversely, the red-sensitive curve illustrates a high degree of variability only at wavelengths below 525 mμ. This would seem to imply that the system is responding primarily to a red-sensitive receptor (maximum 620 mμ), with a small and variable contribution by the blue-green system. These two maxima observed in *sarae* are the same as those reported for *erato* (Swihart, 1964).

By comparison, the single type of spectral efficiency curve characteristic of *Agraulis* demonstrates a fairly uniform variability through-

out the spectrum. This would seem to indicate that the mechanism is not "dominated" by a single receptor system, but rather reflects the neural summation of the activity in several receptor types. Indeed, the peak of such a curve may not correspond to any specific type of receptor, but, rather, indicate a region where the overlapping sensitivity of two receptors "summates" to produce an "artificial" peak. It is, therefore, interesting to note that the peak of the *Agraulis* curve lies midway between the maxima of the two receptors postulated for *sarae* and *erato*.

Extending our analysis to the other forms studied, we find that *ricini* is similar in all respects to *sarae*. This is not particularly surprising when one considers both the extremely close phylogenetic relationship between the two forms (Emsley, 1965) and the basically similar wing coloration (i.e., forewing yellow bands).

Turning to the two similar green forms, *Philaethria dido* and *Victorina steneles,* we find several significant differences from the species previously considered. First is the rather obvious shift in the peak from the 528 mμ region to about 570 mμ. The very small variance between individuals at the peak of the curve suggests that this is probably due to a difference in receptors rather than a mechanism such as that postulated for *Agraulis.*

The second remarkable feature of the *Victorina* curve is the extremely attenuated response to long wavelengths. It seems most unlikely that the orange color of the cornea could be responsible for the diminished responsiveness to long wavelengths. While it is possible that this portion of the curve represents the sensitivity of the receptors, it seems much more probable that some other mechanism is involved (e.g., inhibition by a red receptor system).

It is difficult to extend the preceding type of analysis to *Morpho,* since the responses of this form (ERG and neural) are so different from the preceding forms as to make a direct comparison difficult, if not impossible. Regardless of the final interpretation of the nature of the visual mechanisms of this form, it is clear that virtually all the recorded responses demonstrate a maximum sensitivity to the blue portion of the spectrum. It is obvious that this organism must possess a blue-sensitive receptor. The question is, therefore, whether this represents a unique type of receptor. The neural responses of the other forms have given but little indication of any special sensitivity to short wavelengths. Only *Victorina's* neural spectral efficiency curve demonstrates the type of variability between individuals which could be interpreted as being

clearly indicative of the activity of a blue sensitive system. On the other hand, spectral efficiency curves based upon the ERG B-wave demonstrate a rather considerable sensitivity to short wavelengths. This is true even in the case of the primarily red-sensitive *erato* (Swihart, 1963). It seems possible, therefore, that such a system may be fairly commonly distributed among the Nymphalids; however, evolutionary adaptation has resulted in its contributing little or nothing to the excitation of the information pathway in the vast majority of species where the primary wing coloration is in the long wavelength portion of the spectrum.

To conclude this discussion, it is interesting to speculate as to the evolutionary forces which have contributed to the development of the weak and variable responses characteristic of the forms with the yellow forewing bands, i.e., *sarae* and *ricini.* In considering this problem, one must remember that there are two important factors which have played a role in the development of butterfly wing coloration. These are: protective (warning or mimetic) coloration and the conservative force of sexual selection. These two forces are frequently antagonistic. In fact, the opposing pressures of these two factors are believed (Brower, 1963) to have produced the multiple cases of sexual dimorphism in wing coloration found in butterflies.

As previously noted, many of the primitive Heliconiinae are primarily orange in color. This coloration appears to be due to a pterin pigment (Baust, 1967). A small modification of this molecule has produced the erythropterin pigmentation found in the red spots of *erato,* etc. It seems not unlikely that this refinement of the chromophore, which has produced a coloration with greater purity, has allowed the refinement of highly specific behavior patterns based upon the releasing value of this striking color.

On the other hand, the yellow pigmentation of *sarae* and *ricini* represents the development of an entirely new type of pigment (an amino acid, Brown, 1965) probably in response to some other pressure. It is certain that these forms have not lost the ability to synthesize a pterin pigment since almost all the Heliconiinae demonstrate minute red spots at the base of the forewing. Employment of the yellow pigment vice the red is hard to explain in terms of sexual selection since the low color purity of this pigment would make an inherently poor sign stimulus. On the other hand, the yellow reflects about 207% more light in the visible spectrum than does the red. It seems certain this more brilliant pigmentation is considerably more effective as warning coloration. The species has had to ac-

commodate to this increased emphasis on warning coloration with a lessened dependence on wing coloration as a courtship releaser. This change appears to be reflected in the less specific neural adaptation of the visual pathway to the organism's wing coloration.

It must be admitted that many of the ideas which have been put forth must remain in the category of speculation. To some extent, this seems to be an inherent penalty for attempting to penetrate the perceptual world of another species.

SUMMARY

Specimens of six species of butterflies were examined using standard electrophysiological techniques. Spectral efficiency curves were constructed for each species. For five of the species, this was done on the basis of the magnitude of long-latency, negative polarity, summated potentials, associated with the activity of higher order neurons in the vicinity of the medulla interna.

Two species with yellow forewing spots (*Heliconius sarae* and *H. ricini*) produced similar results, i.e., individuals yielded one of two types of curves, one a non-specific curve peaking in the green, and another peaking in the red, with a shape very similar to the spectral reflectance of the yellow wing pigmentation. The orange butterfly, *Agraulis vanillae,* produced a single type of curve peaking in the orange. The two green butterflies, *Philaethria dido* and *Victorina steneles,* produced curves peaking in the green. The blue butterfly, *Morpho peleides,* produced very different electrical responses. The electroretinogram did not demonstrate the components, which in the preceding forms, are associated with a red-receptor system. The spectral efficiency curve based upon *Morpho's* ERG B-wave demonstrated a maximum in the blue.

On the basis of these observations, and previous studies of a form with red markings (*Heliconius erato*), it is suggested that butterflies possess a neural mechanism which "selects" the output from various receptors in such a manner so as to make the visual system respond maximally to stimulation with colors approximating the wing pigmentation.

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